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Hypolipidemic effect of polysaccharides from *Fortunella* margarita (Lour.) Swingle in hyperlipidemic rats

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ABSTRACT

The objective was to investigate the hypolipidemic effect of polysaccharides from *Fortunella*margarita (Lour.) Swingle (FMPS) in hyperlipidemic rats and the comparative relationship between *in vitro* and *in vivo*. After FMPS feeding, the body weight, liver and spleen index of the hyperlipidemic rats decreased significantly, in a dose-dependent manner. The content of triglyceride, total cholesterol, low density lipoprotein and serum non-esterified fatty acid decreased, and high density lipoprotein, and serum lipase significantly increased after FMPS feeding in hyperlipidemic rats. Notably, high-dose FMPS, exhibited effective hypolipidemic activity, as compared with that of simvastatin. Moreover, histopathological micrographs of hepatic tissue and blood vessel morphology indicated that the fat deposition in liver cells decreased, and the vascular endothelial cells were protected by FMPS. Furthermore, the activities of superoxide dismutase, total antioxidant capacity, glutathione peroxidase, and glutathione-S-transferase were enhanced, and the content of malondialdehyde was decreased by FMPS feeding in the hyperlipidemic rats. A concentration-dependent response was observed. Similarly to the hypolipidemic effect observed *in vitro*, the hypolipidemic effect of FMPS in hyperlipidemic rats was achieved by decreasing the lipid content and enhancing the activity of antioxidant enzymes. Thus, FMPS had a major role in regulating the lipid metabolism disorder in hyperlipidemic rats.

1. Introduction

Hyperlipidemia, a disorder of lipid metabolism, is the major risk factor for coronary heart disease, myocardial infarction, sudden cardiac death and other diseases. It accelerates systemic atherosclerosis by causing occult, progressive, systemic and organic damage to the body (Pappa et al., 2019). Because of its association with secondary cardiovascular and cerebrovascular diseases, as well as severe damage, it results in high morbidity, high fatality and high disability rates; thus, strongly threatening human health and life. Every year, approximately 12 million people worldwide die from cardiovascular diseases and strokes caused by hyperlipidemia (Zhang et al., 2013). Accordingly, finding a means of preventing and controlling hyperlipidemia is particularly important. At present, most hyperlipidemic drugs (simvastatin, lovastatin and acipimox) negatively affect the human body; consequently, the development of natural lipid-lowering compounds in health care products, such as active polysaccharides, have been a widespread goal (Kurbanov et al., 2006; Liao et al., 2018). Some polysaccharides or polysaccharide-rich substances have hypolipidemic effects (Zhang et al., 2013; Zhao et al., 2014).

Free radicals, mainly deriving from biochemical reactions of cells *in vivo*, are atoms, molecules, ions or chemical groups with an unpaired electron. Consequently, free radicals have very high paramagnetic resonance and reactivity, which can destroy the structure and function of proteins, nucleic acids and lipid; thus, resulting in many clinical diseases (Fang et al., 2002). Both *in vivo* and *in vitro* studies have indicated the antioxidant activity of polysaccharides (Zhang et al., 2017). Peduncles of *Hovenia dulcis* polysaccharides exhibit high superoxide radical scavenging activity (Wang et al., 2012). *Camellia sinensis* polysaccharides enhance the activity of superoxide dismutase (SOD) (Xu

Abbreviations: Catalase, (CAT); polysaccharides from *Fortunella* margarita, (Lour.); Swingle, (FMPS); normal control, (NC); hyperlipidemic model, (HM); positive control, (PC); low dose FMPS, (LFP); moderate dose FMPS, (MFP); high dose FMPS, (HFP); total cholesterol, (TC); triglyceride, (TG); low density lipoprotein, (LDL-C); high density lipoprotein, (HDL-C); non-esterified fatty acid, (NEFA); lipase, (LIPA); superoxide dismutase, (SOD); total antioxidant capacity, (T-AOC); glutathione peroxidase, (GSH-Px); glutathione-S-transferase, (GST); malondialdehyde, (MDA); reactive oxygen species, (ROS)

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et al., 2012). *Ganoderma lucidum* polysaccharides increase serum and hepatic SOD activity and are a potential natural product for the treatment of hyperlipidemia-related diseases in rats (Zhao et al., 2012b). Many studies have shown that the pathogenesis of hyperlipidemia is related to overproduction of oxygen free radicals. Yu et al. (2013) have reported that *Rosa laevigata* polysaccharides promote the activity of catalase (CAT), SOD and glutathione peroxidase (GSH-Px) in mice, and decrease the content of malondialdehyde (MAD) effects related to its mechanism of oxidative defense. *Pleurotus eryngii* polysaccharide significantly promotes SOD and glutathione (GSH) enzyme activity in mice, thus, achieving hypolipidemic effects (Chen et al., 2012). *Catathelasma ventricosum* polysaccharides possess antioxidant activity, which may be directly or indirectly responsible for their hypoglycemic and hypolipidemic properties (Liu et al., 2013).

Fortunella margarita (Lour.) Swingle, which originated in southeastern China, is cultivated for its health benefits in many parts of the world, including Europe, Japan, the USA, Brazil and Australia (Zeng et al., 2017b). It is rich in nutrients and bioactive compounds, such as polysaccharides, limonoids and essential oils (Zeng et al., 2014, 2015a). Polysaccharides from FMPS are the main active components, accounting for 9.15 ± 0.13% (g/g) of the dry weight (Zeng et al., 2015b, 2015c). In our previous study, FMPS was found to be a macromolecular hetero-polysaccharide containing four polysaccharide fractions—FMPS1, FMPS2, FMPS3 and FMPS4, isolated with a DEAE Sepharose CL-6B column and Sephadex G-100 gel column—each with different concentrations and structural properties (Zeng et al., 2016). Several studies have reported that the hypocholesteremic and hypolipidemic effects of citrus fruits are attributed to their polysaccharides. Pectic polysaccharides from citrus fruits inhibit the activity of lipase (Espinal-Ruiz et al., 2014). Additionally, polysaccharides from Citrus aurantium and pectin extracted from citrus peel have strong antioxidant activity (Wang et al., 2014). Indeed, FMPS displays strong hypolipidemic effects *in vitro*, on the basis of testing of the pancreatic lipase activity, bile acid-binding ability and antioxidant activity. FMPS1 and FMPS3 display stronger inhibitory effects on pancreatic lipase; FMPS1 and FMPS2 display stronger ability to bind bile acids; and FMPS3 and FMPS4 display greater antioxidant activity. However, few studies have evaluated the hypolipidemic effect of FMPS polysaccharides in cholesterol-fed rats.

Therefore, the objective of this study was to investigate the hypolipidemic effect of polysaccharides from FMPS in hyperlipidemic rats and to determine the comparative relationship between hypolipidemic activity *in vitro* and *in vivo*. The blood lipid indexes (such as triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), non-esterified fatty acid (NEFA), high density lipoprotein (HDL–C), and lipase (LIPA)) in cholesterol-fed rats were determined. Then the liver and vascular pathology of rats were observed through optical microscopy. In addition, the antioxidant indexes (such as SOD, T-AOC, GSH-Px, glutathione-S-transferase (GST), and MDA) in rat plasma and tissues were also measured. Finally, the comparative relationship between the hypolipidemic effects of FMPS *in vitro* and *in vivo* was assessed.

2. Materials and methods

2.1. Preparation of FMPS

F. margarita was provided by the Youxi Agricultural Bureau (Fujian, China). After cleaning and removal of the seeds, fresh *F. margarita* was added to ten volumes of water and crushed in a juicer. The cloudy juice was poured into a beaker and incubated for 2.5 h at 80 °C in a constant temperature water bath (HH-6, Ronghua, Jiangsu, China). The filtrate obtained by passage through nylon cloth (100-mesh sieve), centrifugation (at 2850 × *g* for 15 min) and concentration (at 60 °C for 1 h, RE-52A rotatory evaporator, Yarong, Shanghai, China) was placed into four volumes of ethanol and kept at 25 °C for 24 h. The supernatant was discarded, and the precipitate was recovered and freeze-dried (LG-1.0 vacuum freeze drier, Xinyang, Shenyang, China). The sample was composed mainly of polysaccharides (85.2%, w/w) and protein (8.3%, w/w) (Zeng et al., 2012). High-fat diet was prepared according to the previous method with some modifications (Zeng et al., 2017a). Briefly, it was composed by 89.25% of ordinary diet, 5% of yolk powder, 5% of lard oil, 0.5% of cholesterol, 0.25% of pig bile salts. It was obtained from Wu's animal lab (Wu Experimental Animal Trade Co., Ltd, Fuzhou, China).

2.2. Animals and experimental design

Specific pathogen free Sprague Dawley male rats were purchased from Silaike experimental animal Co. Ltd. (Shanghai, China). The rats were housed in an environmentally controlled room (25 ± 1 °C, (50 ± 5)% humidity, and a 12 day light/dark cycle) and were allowed free access to food and water. All animal studies were performed in compliance with the *Guidelines for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication 85-23, 1996), and all procedures were approved by the Animal Care Review Committee, Fujian University of Traditional Chinese Medicine, China.

The flowchart for study procedures on the hypolipidemic effect of FMPS on rats fed a high-fat diet is shown in Fig. 1. Briefly, after 1 week of acclimation, 72 rats (Body weight, 190.15 ± 12.53 g) were randomly divided into the following 6 groups.

In the normal control (NC) group, 12 rats were fed a normal diet for 6 weeks and administered sterile physiological saline by intragastric administration at a dose of 2 mL/100 g body weight through gavage daily. After the first 2 weeks fed with HFD, the hyerlipidemic diet group rats (60) were took the blood sample from tail vein to measure the lipids. Rats were considered to be heperlipidemia when their serum TC levels had significant difference ($p < 0.05$) compared with control group. Then the heperlipidemic rats were randomly divided into 5 groups of 12 animals each according to the treatment methods, such as: hyperlipidemic model (HM) group, positive control (PC) group, low dose FMPS (LFP) group, moderate dose FMPS (MFP) group, and high dose FMPS (HFP) group. Briefly, the rats in different groups were continued to feed a high-fat diet for 4 weeks and respectively administered sterile physiological saline at a dose of 2 mL/100 g body weight, simvastatin solution at a dose of 4 mg/kg body weight, FMPS solution at a dose of 100 mg/kg body, FMPS solution at a dose of 200 mg/kg body, FMPS solution at a dose of 400 mg/kg body, by intragastric administration through gavage daily for 4 weeks.

2.3. Assay of weight and the viscera index in rats

The weight of rats in each group was measured at the start of the first week, third week and seventh week. Seven weeks later, the rats were killed, and the liver, heart, spleen, kidneys and lungs were rapidly removed, rinsed with pre-cooled saline solution, dried with filter paper and then weighed. The formula for the viscera index is as follows:

$$Viscera\ index = \frac{viscera\ weigh}{rat\ weigh} \quad (1)$$

2.4. Preparation of rat tissue homogenates

The liver, heart, spleen, kidney and lung tissues were rinsed with physiological saline, and then blood was removed and the samples were dried by filter paper. One gram of tissue was added 10 mL sterile physiological saline and homogenized at 4 °C. The homogenates were centrifuged at 4000 rpm/min for 14 min at 4 °C and stored at −20 °C.

2.5. Assay of serum lipids in rats

All animals except those in the NC group were given a high fat diet.

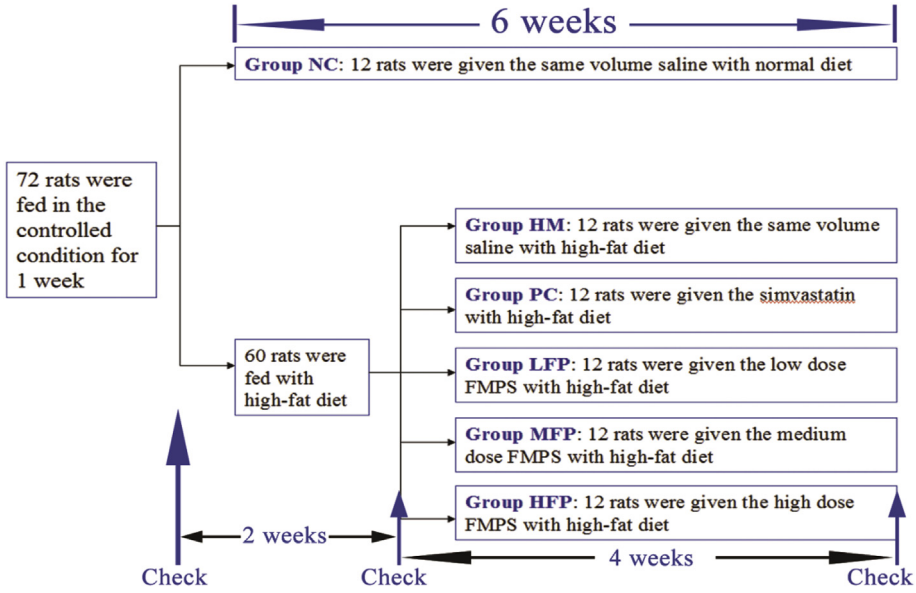

Fig. 1. The flowcharting procedures for studies on the hypolipidemic effect of FMPS on the rats fed a high-fat diet.

After 2 weeks, the rats were in a state of abrosia for 12 h, but were able to have water. Blood samples were collected in heparinized tubes. TG, TC, HDL-C, LDL-C, NEFA and LIPA were measured according to a previous study (Liao et al., 2018; Zhang et al., 2017).

2.6. Pathological observations of liver tissue and blood vessels

Pathological observations of liver tissue and blood vessels after 6 weeks were performed according to a previous study (Zeng et al., 2012), with slight modifications. The liver tissue was cut into three small pieces (5 mm) and fixed with 10% neutral formalin. The blood vessels were fixed with 10% neutral formalin; washed for 90 min with 75% ethanol, 95% ethanol and 100% ethanol three times; and washed 60 min with 100% dimethylbenzene twice. The wax was baked for 2 h at 60 °C, and the process was repeated twice. Samples were observed under a microscope after implantation, sectioning (5 mm) and HE staining.

2.7. Assay of antioxidant indexes in rats

The activity of SOD measured at 550 nm, T-AOC measured at 520 nm, GSH-Px measured at 412 nm, GST measured at 412 nm and MDA measured at 532 nm was determined according to previous studies (Li et al., 2018; Pappa et al., 2019; Wang et al., 2018). The formulas for activity of SOD, T-AOC, GSH-Px, GST and MDA in the plasma and viscera were as follows:

$$SOD\ activity\ in\ plasma\left(U/mL\right)=\frac{OD_1-OD_2}{OD_1}\div 50\%\times A\times B \tag{2}$$

In formula (2), OD_1 is the normal OD value, OD_2 is the measured OD value, A is the reaction system dilution time, and B is the sample dilution time.

$$SOD\ activity\ in\ viscera\left(U/mgprot\right)=\frac{OD_1-OD_2}{OD_1}\div 50\%\times\frac{V_1}{V}\div c \tag{3}$$

In formula (3), OD_1 is the normal OD value, OD_2 is the measured OD value, V_1 is the total volume of reaction liquid, V is the sample quantity, and c is the sample protein concentration.

$$T-AOT\ activity\ in\ plasma\left(U/mL\right)=\frac{OD_1-OD_2}{0.01}\div 30\times\frac{V_1}{V}\times A \tag{4}$$

In formula (4), OD_1 is the normal OD value, OD_2 is the measured OD value, V_1 is the total volume of reaction liquid, V is the sample quantity, and A is the sample dilution time.

$$T-AOC\ activity\ in\ viscera\left(U/mgprot\right)=\frac{OD_1-OD_2}{0.01\times 30}\times\frac{V_1}{V}\times A\div c \tag{5}$$

In formula (5), OD_1 is the normal OD value, OD_2 is the measured OD value, V_1 is the total volume of reaction liquid, V is the sample quantity, A is the sample dilution time, and c is the sample protein concentration.

$$GSH-Px\ activity\ in\ plasma\left(U/mL\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 20\times A\times B \tag{6}$$

In formula (6), OD_1 is the normal OD value, OD_2 is the measured OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, A is the reaction system dilution time, and B is the sample dilution time.

$$GSH-Px\ activity\ in\ viscera\left(U/mgprot\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 20\times A\div t\div c \tag{7}$$

In formula (7), OD_1 is the normal OD value, OD_2 is the measured OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, A is the reaction system dilution time, and c is the sample protein concentration.

$$GST\ activity\ in\ plasma\left(U/mL\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 20\times A\div t\div 0.1 \tag{8}$$

In formula (8), OD_1 is the normal OD value, OD_2 is the measured OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, A is the reaction system dilution time, and t is the reaction time.

$$GST\ activity\ in\ viscera\left(U/mgprot\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 20\times A\div 10\div 0.1\div c \tag{9}$$

In formula (9), OD_1 is the normal OD value, OD_2 is the measured OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, A is the reaction system dilution time, and c is the sample protein concentration.

$$MDA\ activity\ in\ plasma\left(\frac{nmol}{mL}\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 10\times A \quad (10)$$

In formula (10), OD_1 is the measured OD value, OD_2 is the normal OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, and A is the sample dilution time.

$$MDA\ activity\ in\ viscera\left(\frac{nmol}{mgprot}\right)=\frac{OD_1-OD_2}{OD_3-OD_4}\times 10\div c \quad (11)$$

In formula (11), OD_1 is the measured OD value, OD_2 is the normal OD value, OD_3 is the standard OD value, OD_4 is the blank OD value, and c is the sample protein concentration.

2.8. Statistical analysis

SPSS version 16.0 (Chicago, IL, USA) was used for variance analysis and multiple comparisons, and the experimental data are expressed means ± SD. The differences between pairs of groups were analyzed by t-test ($p < 0.05$).

3. Results

3.1. Effects of the high-fat diet on the lipid profiles in rats (after feeding for 2 weeks)

The effects of the high fat diet (after feeding for 2 weeks) on the blood lipid indexes of the rats are shown in Table 1. After 2 weeks, the values of TG, TC, LDL-C, NEFA and LIPA in the model group were significantly higher ($p < 0.05$) than those in the NC group, whereas the HDL-C value was significantly lower ($p < 0.05$) in the model group than the NC group. Those data suggested that the model of hyperlipidemia in rats was established. Moreover, there were no significant ($p > 0.05$) differences in TG, TC, HDL-C, LDL, NEFA and LIPA among the HM, PC, LFP, MFP and HFP groups.

3.2. Effects of FMPS on body weight and the viscera index of hyperlipidemic rats

As shown in Table 2, at the beginning of the study, the body weight of each group was not significantly different ($p > 0.05$). However, the weight of the rats after 2 weeks' feeding, in the high-fat diet group was significantly higher ($p < 0.05$) than that in the NC group. After treatment for 7 weeks, compared with the HM group, the PC, LFP, MFP and HFP groups showed significantly lower weights ($p < 0.05$). The weight of the hyperlipidemic rats decreased with the addition of polysaccharides, in a dose-dependent manner. The effect of FMPS on the viscera index in hyperlipidemic rats is shown in Table 3. After long-term feeding of a high-fat diet, the index of the liver and spleen was significantly ($p < 0.05$) higher than that in normal fed rats. However, no significant effects were observed on the heart, kidney and lung ($p > 0.05$). The liver and spleen indexes of the PC, MFP and HFP groups were significantly lower ($p < 0.05$) than those in the HM group.

Table 2
Effect of FMPS on the body weight of the hyperlipidemic rats (g).

Group	Before experiment (g)	Pre dose (g)	After dose (g)
NC group	188.71 ± 7.03^{a}	259.44 ± 15.6^{b}	268.86 ± 18.98^{d}
HM group	190.62 ± 10.30^{a}	288.64 ± 11.1^{a}	354.39 ± 13.90^{a}
Group PC	190.71 ± 12.22^{a}	283.32 ± 13.2^{a}	311.81 ± 15.47^{bc}
Group LFP	190.35 ± 6.69^{a}	286.78 ± 15.3^{a}	322.39 ± 8.23^{b}
Group MFP	190.80 ± 11.28^{a}	288.19 ± 16.7^{a}	308.48 ± 15.84^{bc}
Group HFP	190.83 ± 9.14^{a}	285.72 ± 14.2^{a}	298.86 ± 9.59^{c}

Different lower case letters (a, b and c) in the same column represent significant differences between different treatments ($p < 0.05$).

3.3. Effects of FMPS on the blood lipid index in hyperlipidemic rats

The effects of FMPS on the TG, TC, HDL-C, LDL-C, NEFA and LIPA values in the hyperlipidemic rats are shown in Fig. 2. The TG, TC, LDL-C, NEFA and LIPA values in the HM, PC, LFP, MFP and HFP groups were higher than those in the NC group, whereas their HDL-C values were lower than that in the NC group before dosing. After dosing, the TG content in the PC, MFP and HFP groups was significantly lower ($p < 0.05$) than that in the NC group. The TG content in the PC, LFP, MFP and HFP groups was lower than that in the HM group ($p < 0.05$), as shown in Fig. 2 (A). The TC and LDL-C content in the PC, LFP, MFP and HFP groups was higher than that in the NC group, but lower than that in the HM group ($p < 0.05$), as shown in Fig. 2 (B) and 2 (D). The hyperlipidemic rats displayed different trends in the values of HDL-C compared with TG, TC and LDL-C in Fig. 2 (C). The HDL-C values in the PC, LFP, MFP and HFP groups were higher than those in the NC and HM groups after dosing. Furthermore, after dosing, the NEFA content in the MFP and HFP groups was not significantly different from that in the NC group, whereas other groups showed levels greater than those in the NC group in Fig. 2 (E). As shown in Fig. 2(F), the LIPA content in the PC, LFP, MFP and HFP groups was significantly higher than those in the NC and HM groups ($p < 0.05$). Among these indexes, the HDL-C, NEFA and LIPA values changed in a dose-dependent manner.

3.4. Pathological observation of liver tissue and blood vessels in hyperlipidemic rats

3.4.1. Pathological observations of liver tissue

The apparent morphology and histopathology (viewed at 100 times magnification) of liver tissue in hyperlipidemic rats is shown in Fig. 3. In the NC group, the hepar was complete (Fig. 3 (A)), whereas in the HM group, it was welled and gray with tiny needle-like particles (Fig. 3 (B)). After treatment, the size, color and texture of hepar markedly improved (Fig. 3 (C), (D), (E), (F)). As shown in Fig. 3 (A), in the hepatocytes were normal with abundant cytoplasm, central nuclei, polygonal shape, and distinct and round cell borders; in addition, the hepatic cords were radial with respect to the central vein in all directions. The major features of hepar in the HM group were hepatomegaly, cabined hepatic sinusoids, blurry hepatic cord inflammatory cell infiltrations, and spotty and patchy necrosis of hepatocytes. The sections of hepatic tissues in the HM group had many large lipid vacuoles (black

Table 1
Effect of the high-fat diet on the lipid profiles in rats (feeding for 2 weeks).

Group	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	NEFA (mmol/L)	LIPA (mmol/L)
NC group	1.60 ± 0.23^{c}	1.61 ± 0.23^{b}	0.82 ± 0.05^{a}	0.25 ± 0.06^{b}	664.14 ± 31.99^{b}	63.57 ± 4.54^{b}
HM group	3.35 ± 0.31^{ab}	2.94 ± 0.09^{a}	0.58 ± 0.08^{b}	1.04 ± 0.13^{a}	1248.57 ± 153.13^{a}	105.00 ± 7.12^{a}
Group PC	3.14 ± 0.31^{b}	2.96 ± 0.39^{a}	0.55 ± 0.13^{b}	1.19 ± 0.24^{a}	1251.57 ± 110.68^{a}	103.86 ± 9.41^{a}
Group LFP	3.31 ± 0.32^{ab}	2.94 ± 0.25^{a}	0.49 ± 0.12^{b}	1.09 ± 0.16^{a}	1220.57 ± 146.09^{a}	99.86 ± 5.61a
Group MFP	3.30 ± 0.29^{ab}	2.89 ± 0.23^{a}	0.47 ± 0.14^{b}	1.05 ± 0.15^{a}	1244.14 ± 129.95^{a}	102.57 ± 2.99^{a}
Group HFP	3.46 ± 0.30^{a}	2.94 ± 0.18^{a}	0.48 ± 0.10^{b}	1.03 ± 0.12^{a}	1267.00 ± 113.83^{a}	106.14 ± 9.06^{a}

Different lower case letters (a, b and c) in the same column represent significant differences between different treatments ($p < 0.05$).

Table 3
Effect of FMPS on the viscera index of the hyperlipidemic rats.

Group	Quantity	Viscera				
		Liver	Heart	Spleen	Kidney	Lung
NC group	9	0.0302 ± 0.0041^{d}	0.0031 ± 0.004^{ab}	0.0029 ± 0.0007^{bc}	0.0040 ± 0.0009^{a}	0.0064 ± 0.0006^{a}
HM group	8	0.0401 ± 0.0029^{a}	0.0027 ± 0.002^{c}	0.0035 ± 0.0008^{a}	0.0032 ± 0.0004^{b}	0.0060 ± 0.0007^{a}
Group PC	9	0.0357 ± 0.0025^{bc}	0.0032 ± 0.003^{ab}	0.0027 ± 0.0004^{bcd}	0.0036 ± 0.0005^{ab}	0.0060 ± 0.0003^{a}
Group LFP	10	0.0378 ± 0.0037^{ab}	0.0029 ± 0.003^{bc}	0.0030 ± 0.0002^{ab}	0.0035 ± 0.0003^{ab}	0.0064 ± 0.0002^{a}
Group MFP	9	0.0363 ± 0.0023^{b}	0.0033 ± 0.004^{a}	0.0023 ± 0.0002^{d}	0.0036 ± 0.0003^{ab}	0.0060 ± 0.0003^{a}
Group HFP	9	0.0329 ± 0.0012^{cd}	0.0033 ± 0.003^{a}	0.0025 ± 0.0002^{cd}	0.0037 ± 0.0004^{ab}	0.0064 ± 0.0006^{a}

Different lower case letters (a, b, c and d) in the same column represent significant differences between different treatments ($p < 0.05$).

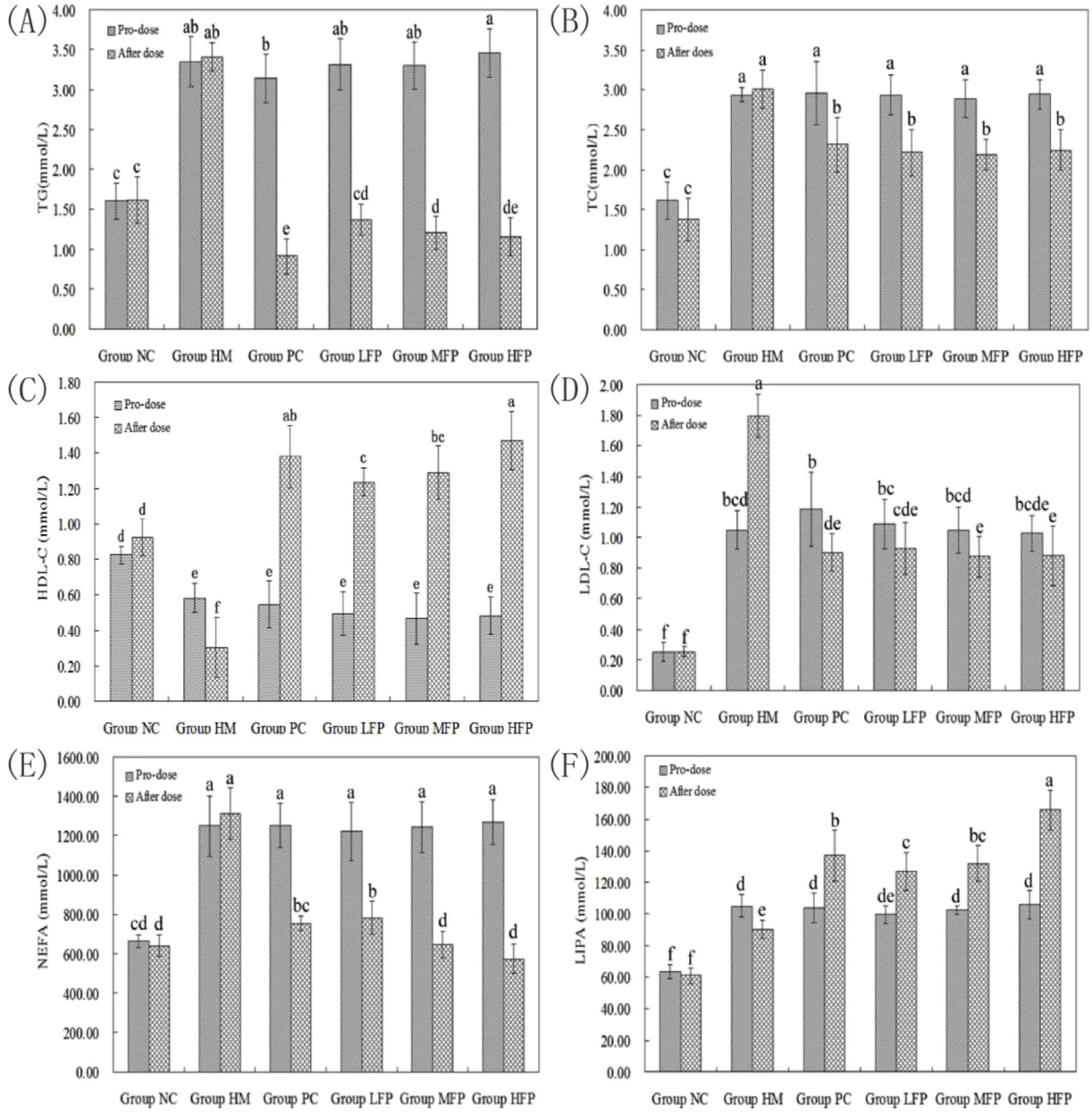

Fig. 2. Effect of FMPS on the TG, TC, HDL-C, LDL-C, NEFA and LIPA values in the hyperlipidemic rats. Different lower case letters in the same chart represent significant differences between different treatments ($p < 0.05$).

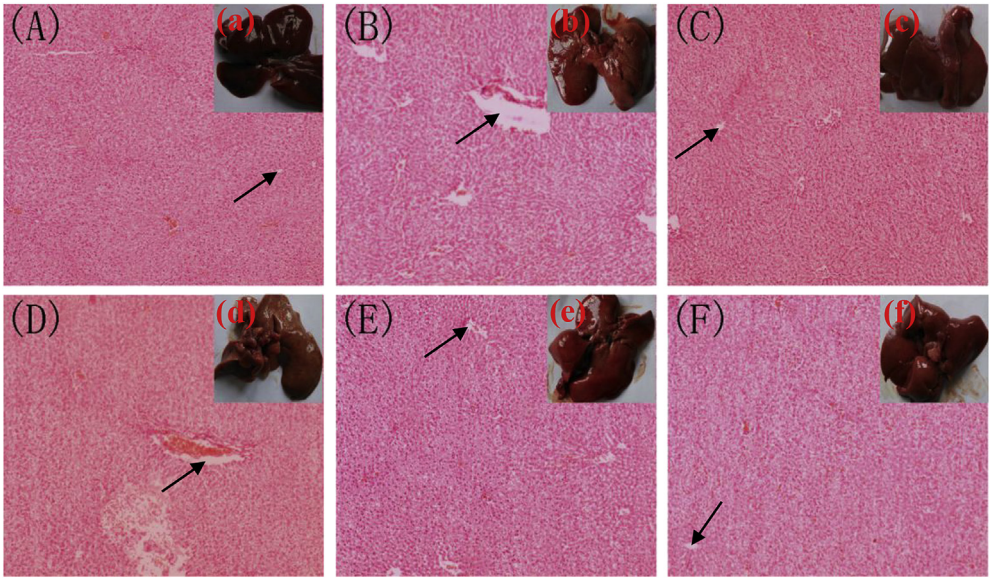

Fig. 3. The apparent morphology ((a): NC group; (b): HM group; (c): Group PC; (d): Group LFP; (e): Group MFP; (f): Group HFP) and histopathological micrographs ((A): NC group; (B): HM group; (C): Group PC; (D): Group LFP; (E): Group MFP; (F): Group HFP) of the hepatic tissue of the hyperlipidia rats.

arrow), and the central vein of the hepatic lobule showed lipid droplets (Fig. 3 (B)). After treatment, the lipid vacuolization decreased, and the accumulation of hepatic lipid droplets was relatively lower in the PC, LFP, MFP and HFP groups (Fig. 3 (C), (D), (E), (F)).

3.4.2. Pathological observations of blood vessels

Hyperviscosity in rats with microscopically observed vascular pathology is shown in Fig. 4. The normal rat vessels were arranged with a smooth intima, and the endothelial cells were intact. There were no lipids or inflammatory cell infiltration in the endothelium, and the structures of the mid-layer and intima were clear. The middle layer was composed of elastic fiber membranes, between which were abundant vascular smooth muscle cells with diamond nuclei and spots of fibrous composition (black arrow in Fig. 4(A)). Nevertheless, the intima in the HM group were incrassated and tumefied. The endothelial cells were partially exfoliated, and the interval broadened. There was evidence of foamy cell infiltration, intima dropsy and tunica media proliferation. The smooth muscle cells migrated to endothelia, with hyperplasia, became deranged and transferred to the endometrium, thus, resulting in histologic changes (black box in Fig. 4(B)). In the PC group, the vascular morphology and structure was improved, and the layers were clear, the intima was smooth, and the structure of endothelial cells was continuous and complete (black arrow in Fig. 4(C)). In the LFP, MFP and HFP groups, the rat blood vessels had different degrees of improvement, especially in the MFP and HFP groups (black arrows in Fig. 4(D), (E), (F)).

3.5. Effects of FMPS on the antioxidant index of hyperlipidemic rats

The effects of FMPS on the activity of serum SOD, T-AOC, GSH-Px, GST and the level of serum MDA of rats in the different experimental groups are shown in Fig. 5. Compared with those in the HM group, the SOD, T-AOC, GSH-Px and GST values in the PC, MFP and HFP groups were significantly higher ($p < 0.05$). These indexes increased with

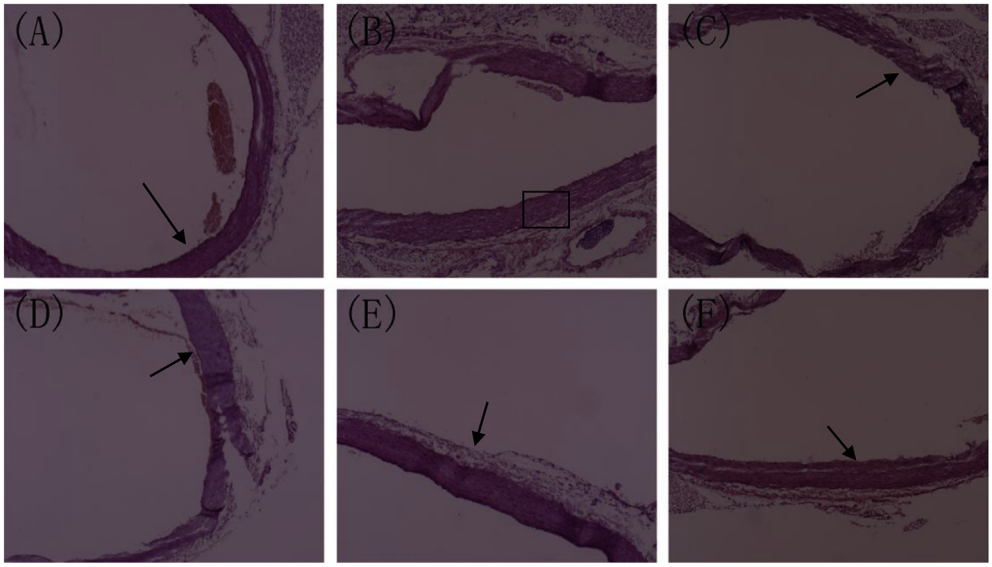

Fig. 4. Histopathological micrographs of blood vessel morphology of the hyperlipidemic rats ((A): NC group; (B): HM group; (C): Group PC; (D): Group LFP; (E): Group MFP; (F): Group HFP).

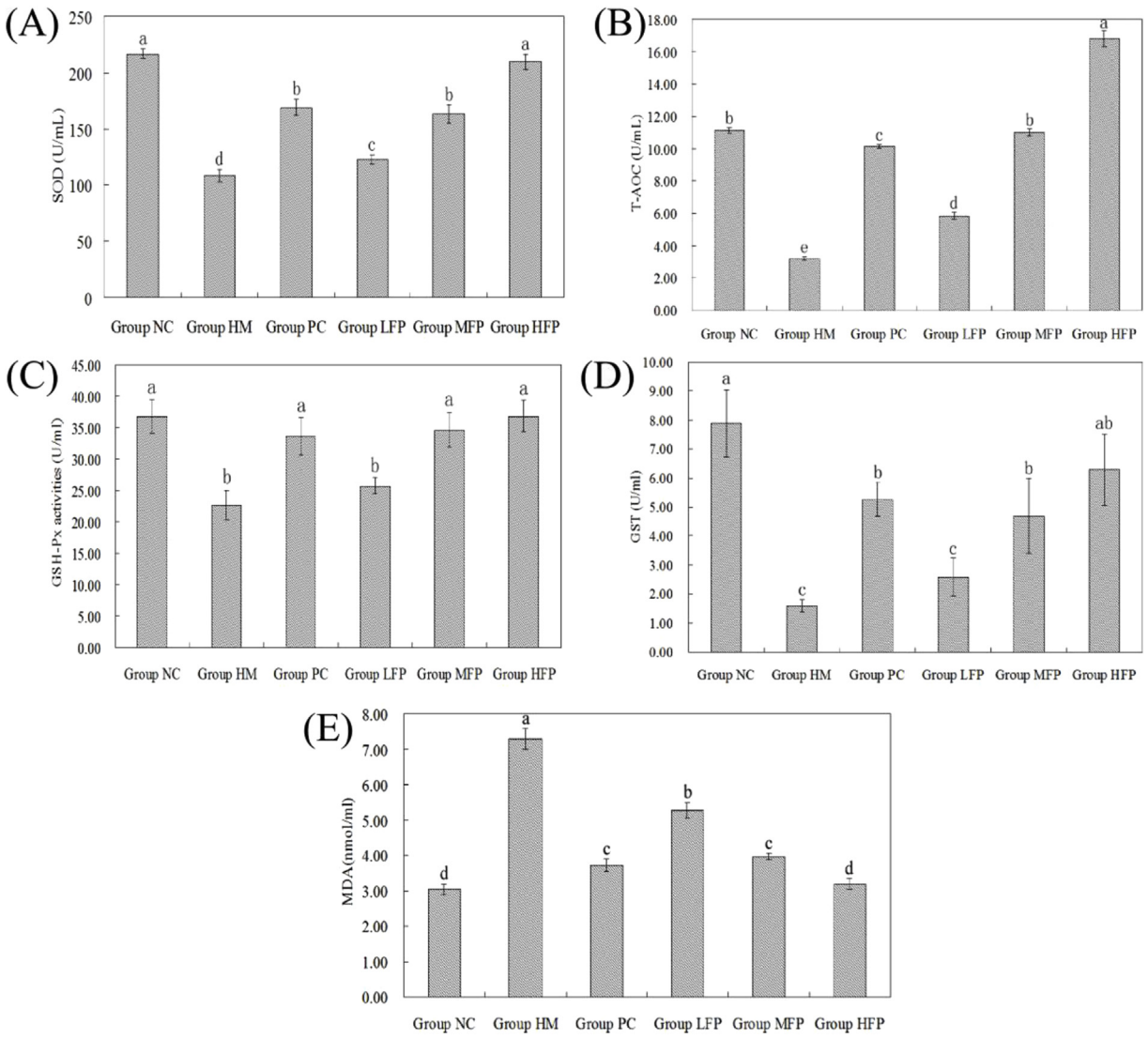

Fig. 5. Effect of FMPS on the antioxidant activity of plasma in the hyperlipidemic rats. Different lower case letters in the same chart represent significant differences between different treatments ($p < 0.05$).

increasing content of FMPS, in a dose-dependent manner, and there were no significant differences between the HFP and NC groups. However, the MDA values in the serum in hyperlipidemic rats declined with increasing content of FMPS, in a dose-dependent manner.

As shown in Table 4, there was a significant decrease ($p < 0.05$) in the activity of organ SOD, T-AOC and GSH-Px, and a significant increase ($p < 0.05$) in the level of viscera MDA in rats in the HM group in comparison with the other groups. These results indicated that administration of HFP significantly increased ($p < 0.05$) the organ levels of SOD. Moreover, the administration of HFP resulted in significant increases ($p < 0.05$) in the liver, heart and kidney levels of T-AOC and GSH-Px. The administration of HFP significantly increased ($p < 0.05$) the level of GST in the heart, but not in the liver, spleen, kidney and lung. The MDA levels were similar in the HFP and NC groups.

4. Discussion

4.1. Blood lipid and pathological observations

Hyperlipidemia includes hypercholesterolemia, hypertriglyceridemia and complex hyperlipidemia. In this study, the levels of TC, TG, LDL-C, LIPA and NEFA increased significantly, many large lipid vacuoles were observed, and the central vein of the hepatic lobule contained lipid droplets. In addition, foam cell infiltration and atherosclerotic plaque formation were observed in histopathologic sections of aortas of hyperlipidemic rats. Serum TC and TG concentrations are positively correlated with the risk of cardiovascular disease, which are known to lead to an increase in blood viscosity (Zhao et al., 2016). TC in the serum mainly exists in LDL-C; when the LDL-C content increases, the arterial intima produces oxygen free radical aggregates and other metabolites (Wang et al., 2015). Additionally, oxygen free radicals can cause inflammation (Baynes, 1991). The LDL in the endothelium is converted to oxidized low density lipoprotein, which plays a key role in the early stages of atherosclerosis (Zhao et al., 2012a). LDL-C, a carrier of TC, transports TC to the endarterium, thus, resulting in the accumulation of cholesterol in intima and formation of foam cells, whereas HDL-C is a carrier involved in reverse transport of TC, and increased HDL-C concentration accelerates the catabolism of TC in the blood (Chen et al., 2011; Kim et al., 2005). There are two main sources of TC and TG in serum. One is exogenous TC and TG absorbed into the alimentary tract and directly absorbed by the small intestine. The other is endogenous TC and TG synthesized by NEFA *in vivo* (Bissonnette et al., 2013). In plasma, TC and cholesterol-COA form cholesterol, the major component of LDL-C and HDL-C in plasma lipoproteins (Van Herpen and Schrauwen-Hinderling, 2008). HDL-C removes excess cholesterol from peripheral tissues and delivers it to liver hepatocytes for

Table 4
Effect of FMPS on the antioxidant abilities of viscera in the hyperlipidemic rats.

Group	Viscera				
	Liver	Heart	Spleen	Kidney	Lung
SOD (U/mgprot)					
NC group	196.19 ± 7.11^{a}	57.71 ± 1.61^{a}	115.03 ± 4.74^{a}	148.85 ± 2.76^{a}	134.09 ± 1.60^{a}
HM group	103.31 ± 2.79^{e}	22.34 ± 2.79^{f}	57.52 ± 2.74^{f}	78.60 ± 4.24^{e}	73.02 ± 5.41^{e}
Group PC	168.42 ± 5.59^{bc}	46.24 ± 1.60^{c}	96.16 ± 1.57^{c}	121.00 ± 1.61^{b}	116.29 ± 2.70^{b}
Group LFP	125.42 ± 5.59^{d}	29.59 ± 1.60^{e}	70.30 ± 1.58^{e}	88.48 ± 3.06^{d}	93.12 ± 2.74^{d}
Group MFP	162.32 ± 4.93^{c}	40.69 ± 1.60^{d}	81.38 ± 5.77^{d}	104.24 ± 4.27^{c}	108.19 ± 7.34^{c}
Group HFP	177.38 ± 2.69^{b}	53.52 ± 1.57^{b}	103.99 ± 2.81^{b}	143.33 ± 8.92^{a}	128.54 ± 4.24^{a}
T-AOC (U/mgprot)					
NC group	25.49 ± 0.31^{b}	31.63 ± 0.88^{b}	36.72 ± 1.01^{a}	25.88 ± 0.37^{bc}	29.26 ± 1.92^{a}
HM group	13.06 ± 0.11^{e}	19.57 ± 0.43^{d}	28.66 ± 0.83^{c}	16.46 ± 0.67^{d}	18.85 ± 0.80^{c}
Group PC	23.43 ± 0.70^{c}	25.19 ± 0.26^{c}	36.17 ± 1.11^{a}	26.90 ± 1.26^{b}	28.07 ± 1.11^{a}
Group LFP	17.23 ± 0.22^{d}	25.02 ± 0.28^{c}	30.73 ± 0.61^{b}	24.29 ± 1.02^{c}	23.80 ± 1.69^{b}
Group MFP	25.27 ± 0.32^{b}	30.89 ± 0.51^{b}	31.10 ± 0.91^{b}	32.59 ± 1.45^{a}	29.85 ± 0.89^{a}
Group HFP	31.75 ± 0.47^{a}	36.80 ± 0.22^{a}	36.96 ± 0.55^{a}	33.32 ± 0.94^{a}	30.30 ± 1.09^{a}
GSH-Px (U/mgprot)					
NC group	494.73 ± 13.89^{b}	423.86 ± 8.14^{b}	524.60 ± 20.17^{a}	455.58 ± 8.99^{b}	477.45 ± 15.00^{a}
HM group	282.83 ± 14.27^{e}	307.04 ± 11.87^{d}	422.16 ± 10.15^{c}	344.82 ± 10.45^{d}	353.46 ± 12.93^{c}
Group PC	442.01 ± 14.99^{c}	446.31 ± 17.16^{b}	545.19 ± 9.98^{a}	472.08 ± 14.69^{b}	483.11 ± 11.08^{a}
Group LFP	368.00 ± 21.98^{d}	338.91 ± 13.48^{c}	449.61 ± 13.92^{b}	406.87 ± 6.17^{c}	431.92 ± 19.99^{b}
Group MFP	474.02 ± 21.59^{b}	423.48 ± 17.56^{b}	535.37 ± 12.22^{a}	462.66 ± 14.82^{b}	469.43 ± 10.74^{a}
Group HFP	555.04 ± 16.80^{a}	525.58 ± 16.28^{a}	546.44 ± 19.47^{a}	496.49 ± 15.04^{a}	485.14 ± 16.49^{a}
GST (U/mgprot)					
NC group	35.44 ± 2.39^{a}	24.27 ± 1.19^{a}	18.37 ± 1.55^{a}	21.98 ± 1.72^{a}	21.08 ± 0.96^{a}
HM group	11.00 ± 1.81^{c}	15.78 ± 1.72^{bc}	8.36 ± 1.00^{d}	13.24 ± 1.17^{c}	14.87 ± 1.81^{c}
Group PC	26.91 ± 3.33^{b}	22.01 ± 2.36^{a}	17.29 ± 1.15^{ab}	23.26 ± 3.09^{a}	21.56 ± 1.45^{a}
Group LFP	15.08 ± 2.22^{c}	14.74 ± 0.57^{c}	15.21 ± 0.60^{c}	15.34 ± 1.04^{bc}	17.42 ± 0.67^{b}
Group MFP	26.03 ± 3.67^{b}	18.28 ± 1.83^{b}	15.82 ± 0.50^{bc}	16.68 ± 2.02^{b}	17.80 ± 1.24^{b}
Group HFP	30.69 ± 2.08^{ab}	23.06 ± 1.23^{a}	16.33 ± 0.60^{bc}	16.85 ± 1.44^{b}	17.93 ± 0.69^{b}
MDA (nmol/mgprot)					
NC group	10.92 ± 0.08^{b}	15.31 ± 0.74^{e}	20.34 ± 0.94^{d}	14.40 ± 0.85^{d}	21.69 ± 1.25^{d}
HM group	19.47 ± 0.30^{a}	23.04 ± 1.76^{a}	30.24 ± 1.59^{a}	23.29 ± 1.03^{a}	30.87 ± 1.33^{a}
Group PC	11.40 ± 0.47^{b}	17.87 ± 0.36^{bc}	22.51 ± 1.73^{cd}	16.52 ± 2.83^{cd}	23.72 ± 1.68^{bcd}
Group LFP	11.59 ± 0.81^{b}	18.89 ± 0.67^{b}	27.87 ± 1.55^{b}	19.81 ± 0.85^{b}	26.09 ± 1.57^{b}
Group MFP	11.55 ± 0.74^{b}	17.39 ± 0.14^{cd}	25.99 ± 0.94^{b}	18.89 ± 1.80^{bc}	25.80 ± 1.38^{bc}
Group HFP	11.11 ± 0.22^{b}	16.38 ± 0.58^{de}	23.53 ± 0.73^{c}	18.60 ± 1.03^{bc}	23.53 ± 1.22^{cd}

Different lower case letters (a, b, c, d and e) in the same column represent significant differences between different treatments ($p < 0.05$).

cholesterol excretion, whereas LDL-C transports cholesterol to peripheral tissues from the liver (Sathivel et al., 2008). Decreased LDL-C indicates that the cholesterol is lowered in the serum, and cholesterol metabolism is increased (Jiang et al., 2015). LIPA, a digestive enzyme secreted by the pancreas, catalyzes the hydrolysis of TC to glycerol and fatty acids (Young, 2001). NEFA are the intermediate products of fat metabolism, and increases in NEFA content can impair capillary circulation (De Jongh et al., 2004). The serum TC, TG, LDL-C and NEFA levels significantly decreased after FMPS administration to hyperlipidemic rats, whereas the serum HDL-C and LIPA levels increased, thus, indicating that FMPS has a potential hypolipidemic effect.

4.2. Antioxidant activity

Many studies have clearly demonstrated the essential role of reactive oxygen species (ROS) in the occurrence and development of hypolipidemic diseases (Zhou et al., 2010). ROS can change the structure of protein, attack DNA and cause lipid peroxidation, thus, resulting the apoptosis of endothelial cells and the proliferation of smooth muscle cells (Li et al., 2007; Sugiura et al., 2011). ROS oxygenate and modify LDL-C, such as MDA, thus, forming MDA-LDL-C and resulting in the accumulation of cholesterol in intima and the formation of foam cells (Ueda et al., 2011). MDA, the final product of lipid peroxidation, inhibits the activity of LIPA and promotes the accumulation of TC (Adewole et al., 2006). The content of MDA, an important indicator of lipid peroxidation level, can be used as a biomarker of oxidative stress in patients with hypercholesterolemia (Yu et al., 2013). GST can decrease the activity of free radicals (Hu et al., 2018; Jang et al., 2010). SOD is the main enzyme responsible for scavenging oxygen free radicals. GSH-Px is an important catalytic hydrogen peroxide decomposing enzyme that is widely found in the body and protects cell membrane structure and function (Park et al., 2002). T-AOC activity reacts to the capacity of the non-enzyme antioxidant defense system in the organ (Zhu et al., 2013). Free radicals with lower activity can be better converted to H_2O_2 by SOD, and GSH-Px decomposes H_2O_2 into H_2O and CO_2, thereby inhibiting the synthesis of MDA in the body. In the present study, the activity of SOD, T-AOC, GSH-Px and GST in the HM group was lower, and MDA was higher, than that in other groups; thus, suggesting the proliferation of smooth muscle cells and the presence of partially exfoliated endothelial cells. Nevertheless, SOD, T-AOC, GSH-Px and GST were significantly elevated after FMPS administration to hyperlipidemic rats, whereas the MDA content was depleted, thus, indicating that FMPS has antioxidant activity.

At present, there are three main mechanisms through which polysaccharides affect lipid metabolism: inhibition of pancreatic lipase activity, binding bile acids and antioxidant activity (Ntchapda et al., 2015; Teng et al., 2013). In our previous study (Zeng et al., 2016), FMPS fractions displayed hypolipidemic activity *in vitro*, including a stronger inhibitory effect on pancreatic lipase, stronger ability to bind bile acids, and greater antioxidant activity. The current study was conducted to explore the relationship between antioxidant activity and hyperlipidemia. On the one hand, a possible hypolipidemic mechanism is that FMPS increases the content of LIPA *in vivo*, thus, accelerating the decomposition of TG and reducing the cholesterol content. On the other hand, FMPS enhances the activity of SOD, T-AOC, GSH-Px and GST in the body, inhibits the formation of lipid peroxides and decreases the

accumulation of cholesterol in cells. In conclusion, FMPS decreases blood lipids in rats, decreases lipid peroxidation of the cell membrane and lowers blood lipids by removing free radicals in the body.

5. Conclusions

The objective of this research was to investigate the hypolipidemic effect of polysaccharides from FMPS in hyperlipidemic rats and the comparative relationship between hypolipidemic activity *in vitro* and *in vivo*. FMPS significantly decreased body weight and the liver and spleen index in hyperlipidemic rats, in a dose-dependent manner. The content of TG, TC, LDL-C and NEFA decreased, and that of HDL-C and LIPA increased significantly after FMPS feeding in hyperlipidemic rats. The low dose of FMPS had a significant hypolipidemic effect, as compared with the lipid levels in the high fat model group. The hypolipidemic effect of high dose FMPS on the serum lipid index in hyperlipidemic rats was equal to or better than that of simvastatin, thus, indicating that FMPS has a substantial regulatory effect toward blood lipid metabolism disorders in hyperlipidemic rats. Moreover, the fat deposition in liver cells decreased, and the vascular endothelial cells were protected by FMPS feeding in hyperlipidemic rats.

Furthermore, FMPS significantly increased the activity of SOD, GSH-Px and GST in the plasma and tissues of hyperlipidemic rats and improved the T-AOC capacity, while significantly decreasing the content of MDA; these defects exhibited a dose-dependent relationship. Compared with the high-fat model group, the low-dose FMPS group showed significantly elevated SOD and T-AOC in the plasma and tissues of hyperlipidemic rats, and significantly decreased MDA content. The content of GSH-Px and GST in plasma and tissues was enhanced by feeding with a moderate dose of FMPS. Similar to the hypolipidemic effect *in vitro*, a hypolipidemic effect of FMPS in hyperlipidemic rats was observed: the lipid content was decreased, and the activity of antioxidant enzymes was enhanced. The results indicated that FMPS has a major role in regulating lipid metabolism disorder in hyperlipidemic rats.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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